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Effect of Plant Growth-Promoting Rhizobacteria Consortium on Morphological and Physiological Parameters and NPK Content in Grain and Straw of Wheat

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A B S T R A C T

The application of PGPR can be implied as a cost-effective alternative to the use of high doses of fertilizers, required for making nitrogen and phosphorus available to the soil for optimum plant growth. A pot Experiment was carried out for two years during rabi season of 2019 and 2020 with wheat cv. HUW-234, thirteen treatments comprising of T_1 (no fertilizer and no PGPR), T_2 (100% NPK), T_3 (N_2 fixer), T_4 (PSB), T_5 (N_2 fixer + PSB), T_6 (50% NP), T_7 (50% NP + N_2 fixer), T_8 (50% NP +PSB), T_9 (50% NP + N_2 fixer +PSB), T_{10} (75% NP), T_{11} (75% NP + N_2 fixer), T_{12} (75% NP + PSB) and T_{13} (75% NP + N_2 fixer + PSB) and three replications. The results showed significant increment in dry matter, leaf area, relative water content and NPK content of grain in T_{13} (75% NP + N_2 fixer + PSB) followed by treatment T_2 (100% NPK). The least of all the above parameters was recorded in T_1 control (no fertilizer and no PGPR). The treatment with PGPR alone and 50% RDF alone also recorded significantly lower values for above parameters as compared to 75% RDF in combination with PGPR treatment. Plant height, leaf area, total dry weight, relative water content and NPK content in wheat were not affected by a 25% decrease in recommended dose of NPK after inoculation with the composite culture of PGPR (*Azotobactor* + *Pseudomonas* + *B. Poymyxa*). Hence, these bacterial isolates allow the use of lower than recommended dose of fertilizer and can be recommended for sustainable soil health.

***Key words*:** Wheat, N_2 fixer (*Azotobacter chroococcum*), PSB (*Pseudomonas putida* + *Bacillus polymyxa*), NPK, Relative water content

Wheat (*Triticum aestivum* L.) has been cultivated since prehistoric times around the world. It belongs to the Graminae family, an important staple food crop not only in India but all over the world. It occupies a unique position in human life as it is the primary source of food and energy along with a large number of end-use products like chapati, bread, cookies, and pasta. Wheat is grown on 220.4 million hectares worldwide, producing 765.4 million tonnes of cereals per year [1]. Wheat in India is grown on an area of 28.5 million hectares producing 87.5 million tonnes [1].

The North West Plains Zone and the North East Plains Zone which includes the states of Punjab, Haryana, Uttar Pradesh, Bihar and Rajasthan are the major wheat producing regions of the country, producing 80% of the total wheat. *Triticum aestivum* (bread wheat) accounts for about 95% of the wheat grown in the country. The growth and yield of a plant are determined by the availability of certain specific mineral nutrients which are absolutely essential for the completion of its life cycle [2]. This is why the supply of these essential nutrients (in particular nitrogen, phosphorus and potassium) to plants in the form of chemical fertilizers is an integral part of intensive agriculture. Population growth is putting immense pressure on agricultural land for more crop yields, resulting in the increasingly intensive use of chemical fertilizers. However, these soil amendments are not only expensive but also considered a potential source of environmental pollution. Moreover, the potential of chemical fertilizers to increase crop yields has already been tapped around the world. Today, it may not be possible to further extend the burden of using chemical fertilizers. Thus, effective additional technologies should be exploited to obtain more crop yields [3]. The application of PGPR as biofertilizers and biological control agents is considered as an alternative or complementary way to reduce the use of chemicals in agricultural production [4,5,6]. Soil microbial communities in the rhizosphere contribute to plant growth by recycling nutrients and making them available [7], improving root health by competing with root pathogens [8] or by increasing the absorption of nutrients [9].

Nitrogen and phosphorus are major plant nutrients and can be supplied to plants by inoculating with effective N_2 scavengers and phosphate solubilizing microorganisms (PSM),

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respectively, into the soil. During intergeneric interaction, N_2-fixing microorganisms supply N to plants and thereby improve soil N status [10].

Bio-fertilizer is a substance that contains living micro-organisms which, when applied to seeds, plant surfaces or soil, colonize the rhizosphere or interior of the plant and promote growth by increasing the supply or availability of primary nutrients to the host plant [5]. Through the use of bio-fertilizers, healthy plants can be grown, while improving the sustainability and health of the soil. Since they play multiple roles, a preferred scientific term for these beneficial bacteria is "plant growth-promoting rhizobacteria" (PGPR). Bio-fertilizers provide environmentally friendly organic agricultural inputs and are more cost effective than chemical fertilizers, such as Rhizobium, Azotobacter, Azospirilium and BGA have been used for a long time.

MATERIALS AND METHODS

A pot experiment was conducted in the polyhouse at Agricultural Farm, Institute of Agricultural Sciences, BHU, Varanasi for two years i.e., in Rabi 2019 and 2020. Wheat cv.HUW-234 widely grown and recommended for late sown condition in Eastern Uttar Pradesh was used for the study. The experiment comprised thirteen treatments viz. T_1 (no fertilizer and no PGPR), T_2 (100% NPK), T_3 (N_2 fixer), T_4 (PSB), T_5 (N_2 fixer + PSB), T_6 (50% NP), T_7 (50% NP + N_2 fixer), T_8 (50% NP + PSB), T_9 (50% NP + N_2 fixer + PSB), T_{10} (75% NP), T_{11} (75% NP + N_2 fixer), T_{12} (75% NP + PSB) and T_{13} (75% NP + N_2 fixer + PSB). All treatments were replicated thrice in a completely randomized block design. Urea, single super phosphate (SSP) and muriate of potash were used as source of chemical fertilizer, whereas, *Azotobacter chroococcum* was used as N_2 fixer and mixture of *Pseudomonas putida* and *Bacillus polymyxa* were used as phosphate solubilizing bacteria.

Seed of wheat was obtained from Department of Genetics and Plant Breeding, BHU, Varanasi. The experiment was carried out in pots of 10 kg soil capacity. The soil was mixed with recommended dose of fertilizers (RDF); N: P: K @120:60:60 $kgha^{-1}$ as well as 50 per cent and 75 per cent of the recommended dose as per the treatment. Bio-fertilizer was given as seed treatment after seed surface sterilizing with 0.1% $Hgcl_2$ for 2 min and rinsed five times with sterilized water. Pure culture of *Azotobacter chroococcum* (N_2 fixer), *Pseudomonas putida* and *Bacillus polymyxa* were grown in nutrient broth by incubation at 120 rpm at 30°C for 4 days. Healthy seeds weighed for each pot were separately inoculated as per treatments in plastic bags with 2 ml of each culture of 4 days old broth cultures grown in specific media of respective inoculants along with 10 ml of 1% (w/v) sticker solution of gum acacia to ensure bacterial population in the range of 10^7 to 10^8 cfu per seed. After drying for one hour in shade, uninoculated seeds were sown first followed by inoculated seeds just to avoid contamination. All the treatments except T_1 was supplied with 100% K fertilizer.

Morphological and physiological were recorded at 30 days after sowing (DAS), 60 DAS and 90 DAS except NPK contain (grain and straw at harvest). The plant height was measured at 30, 60 and 90 DAS and expressed in centimeters. Leaf area at 30, 60 and 90 DAS was measured by leaf area meter by taking 5 middle and average-sized leaves from each replication. The average value of five leaves was recorded. Relative water content was estimated by using formula and nitrogen in grain and straw was obtained by Kjeldahl method [11], Phosphorus by Vandomolybdate phosphoric yellow colour method [11] and Potassium was determined flame photometrically [12]. The analysis of variance for the completely randomized design was employed and critical differences were calculated at 5% probability level. If the variance ratios (F-test) were found to be significant, the standard error of mean (SEm±) and critical difference (CD) were calculated accordingly.

RESULTS AND DISCUSSION

Effect of plant growth-promoting rhizobacteria consortium on morphological parameters in wheat

The pooled data pertaining to effect of PGPR consortium on plant height and leaf area in wheat is presented in (Table 1). The plant height was recorded highest in treatment T_2 **(**100% NPK**)** at two growth stages, i.e., 30 DAS and 60 DAS whereas, at 90 DAS, treatment T_{13} (75% NP + N_2 fixer + PSB**)** recorded the highest plant height. The least plant height was recorded in control **(**no fertilizers and no PGPR**)** during all the growth stages. T_{13} (75% NP + N_2 fixer + PSB) at 90 DAS did not differ significantly with T_2 (100% NPK) for plant height. The treatment with PGPR alone and 50% RDF alone recorded lesser plant height as compared to 75% RDF and fertilizer doses in combination with PGPR treatment. The treatment combination differed significantly for plant height at all the growth stages. Similar increases in plant height and spike length of wheat plants inoculated with the PGPR consortium has been reported due to alteration in distribution patterns of assimilates in plants and also affect on the growth pattern [13].

The results showed that leaf area was significantly higher in treatment T_{13} (75% NP + N_2 fixer + PSB) at all the growth stages, i.e., 30 DAS, 60 DAS and 90DAS. The values in T_{13} was followed by T_2 (100% NPK) at 60DAS but at two growth stages i.e., 30DAS and 90 DAS T_{13} was followed by T_{12}. However, minimum values was obtained in control (no fertilizers and no PGPR) in pooled data of both the years. The treatment with PGPR alone and 50% RDF alone recorded lesser leaf area as compared to 75% RDF and fertilizer doses in combination with PGPR treatment. The treatment combination differed significantly for leaf area at all the growth stages**.** A similar finding was also reported by [14] observed that the application of Azospirillum to wheat had a significant effect on plant height, number of tillers, leaves, ears, flag leaf area (cm^2) and dry weight. Similarly, the growth attribute of wheat, i.e., shoot length, root length, shoot fresh weight and dry weight, root fresh and dry weight, chlorophyll content, leaf number, yield parameters and mineral content (NPK) of wheat increased with PGPR [15].

Effect of plant growth-promoting rhizobacteria consortium on physiological parameters in wheat

The effect of PGPR alone and in consortium on total dry matter and relative water content in wheat is presented in (Table 2). The pooled data of two years showed the dry weight was significantly higher in treatment T_{13} (75% NP + N_2 fixer + PSB**)** at all the growth stages, i.e., 30 DAS, 60 DAS and 90 DAS. However, least values were obtained in control **(**no fertilizers and no PGPR**)** in both the season of experimentation. T_2 (100%) recorded the second highest dry matter at all the growth stages during both the years. The treatment with PGPR alone and 50% RDF alone recorded least dry matter as compared to 75% RDF and fertilizer doses in combination with PGPR treatment. The treatment combination differed significantly for total dry matter at all the growth stages during both the years. [16] have reported that higher level of dry matter production could be obtained mainly due to higher enzyme activities in the rhizosphere and

better availability of nutrient in addition to the production of nutrient regulators for plant growth. Root and shoot weight (both fresh and dry) were found higher when PGPR were applied along with chemical fertilizers. Production of phytohormones especially auxin (IAA) helps in root development [17].

Table 1 Effect of plant growth promoting rhizobacteria consortium on morphological parameters in wheat (*Triticum aestivum* L.) at different growth intervals during Rabi 2019 and Rabi 2020 (Pooled data)

Treatments	Plant height (cm)			Leaf area (cm^2)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1: Control (no fertilizers and no PGPR)	27.78	51.34	56.95	202.05	249.35	202.10
T_2: 100% NPK	41.52	72.88	74.38	235.65	281.75	235.68
T_3: N_2 fixer (*Azotobactor*)	36.15	69.78	64.62	205.65	252.55	205.70
T_4: PSB (*Pseudomonas* + *Bacillus polymyxa*)	38.25	70.42	62.92	208.95	255.50	208.99
T_5: N_2 fixer (*Azotobactor*) + PSB (*Pseudomonas* + *B. polymyxa*)	38.82	67.15	66.08	211.15	258.15	211.42
T_6: 50% NP	36.32	64.98	68.29	213.25	259.50	213.27
T_7: 50% NP + N_2 fixer (*Azotobactor*)	37.02	67.75	69.75	216.55	262.90	216.59
T_8: 50% NP + PSB (*Pseudomonas* + *Bacillus polymyxa*)	39.38	67.82	71.63	221.35	267.55	221.37
T_9: 50% NP + N_2 fixer (*Azotobactor*) + PSB (*Pseudomonas* + *Bacillus polymyxa*)	39.72	69.45	70.77	229.35	275.30	229.39
T_{10}:75% NP	40.12	70.95	72.12	227.95	273.95	227.99
T_{11}: 75% NP + N_2 fixer (*Azotobactor*)	40.32	70.98	72.82	233.95	279.25	233.99
T_{12}: 75% NP + PSB (*Pseudomonas* + *Bacillus polymyxa*)	40.55	70.42	73.48	236.15	285.10	236.39
T_{13}: 75% NP + N_2 fixer (*Azotobactor*) + PSB (*Pseudomonas* + *Bacillus polymyxa*)	40.58	70.32	75.05	254.15	302.00	254.38
S. Em (±)	0.44	0.53	0.34	0.63	1.19	0.65
C.D. at 0.5%	1.30	1.57	1.01	1.83	3.48	1.91

Table 2 Effect of plant growth promoting rhizobacteria consortium on physiological parameters in wheat (*Triticum aestivum* L.) at different growth intervals during Rabi 2019 and Rabi 2020 (Pooled data)

Treatments	Total dry matter (g)			RWC (cm^2)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1: Control (no fertilizers and no PGPR)	2.92	4.08	5.00	69.03	71.53	73.00
T_2: 100% NPK	7.15	10.25	13.00	87.50	88.42	89.33
T_3: N_2 fixer (*Azotobactor*)	3.17	4.35	5.30	70.67	73.80	76.53
T_4: PSB (*Pseudomonas* + *Bacillus polymyxa*)	3.50	4.67	5.98	72.07	76.10	78.33
T_5: N_2 fixer (*Azotobactor*) + PSB (*Pseudomonas* + *B. polymyxa*)	3.94	5.07	6.37	73.87	76.93	79.42
T_6: 50% NP	4.34	5.77	6.82	75.23	77.87	81.50
T_7: 50% NP + N_2 fixer (*Azotobactor*)	4.87	6.68	7.45	76.73	80.92	83.13
T_8: 50% NP + PSB (*Pseudomonas* + *Bacillus polymyxa*)	5.15	7.35	7.97	78.67	82.00	84.80
T_9: 50% NP + N_2 fixer (*Azotobactor*) + PSB (*Pseudomonas* + *Bacillus polymyxa*)	5.70	8.44	9.25	82.47	84.90	86.62
T_{10}:75% NP	5.77	8.18	8.60	82.43	85.17	87.22
T_{11}: 75% NP + N_2 fixer (*Azotobactor*)	6.52	8.80	11.07	84.78	87.22	87.98
T_{12}: 75% NP + PSB (*Pseudomonas* + *Bacillus polymyxa*)	7.00	9.89	11.60	86.53	88.72	89.40
T_{13}: 75% NP + N_2 fixer (*Azotobactor*) + PSB (*Pseudomonas* + *Bacillus polymyxa*)	7.63	10.60	13.49	90.02	92.00	93.67
S. Em (±)	0.07	0.07	0.14	0.29	0.37	0.31
C.D. at 0.5%	0.22	0.21	0.43	0.86	0.95	0.92

The impact of varying NPK levels with different combination of PGPR on relative water content at 30, 60 and 90 DAS in wheat crop was recorded. The pooled data showed that relative water content was significantly higher in treatment T_{13} (75% NP + N_2 fixer + PSB**)** followed by T_2 (100% NPK**)** at 30 DAS but at 60DAS and 90DAS, T_{13} was followed by T_{12}. However, the least values was obtained in control (no fertilizers and no PGPR) during all the growth stages**.** The treatment with PGPR alone and 50% RDF alone recorded the lowest relative water content as compared to 75% RDF and fertilizer doses in combination with PGPR treatment. The treatment combination differed significantly for relative water content at all the growth stages. A similar finding was also reported when PGPR studies were performed by measuring the relative water content (RWC) in water-stressed wheat plants either inoculated or not inoculated with a beneficial microorganism [18]. Higher shoot length, root length, shoot dry weight, root dry weight and RWC were also measured in the PGPR-inoculated wheat plant under mercury toxicity [19].

Effect of plant growth-promoting rhizobacteria consortium on NPK content in grain and straw of wheat

The pooled data with respect to effect of different doses of chemical fertilizer in combination of PGPR on NPK content in grain and straw of wheat is presented in (Table 3). The results revealed that nitrogen content in grain was significantly higher in treatment T_{13} **(**75% NP + N_2 fixer + PSB**)** during both the years. Likewise, the N content in straw was also maximum in T_{13} (75% NP + N_2 fixer + PSB) which was significantly at par with T_2 (100% NPK) and T_{11}. Moreover, the least values was obtained in control (no NPK and no PGPR) in both grain and straw. The phosphorus content in grain and straw of wheat increased with increasing fertility level during both the years.

The results revealed that phosphorus content in grain was significantly higher in treatment T_{13} (75% NP + N_2 fixer + PSB) which was at par with T_2 (100% NPK). Likewise, the phosphorus content in straw was also maximum in T_{13} which was significantly followed by T_2 (100% NPK). Moreover, least values was obtained in control in grain and straw respectively. The results revealed that potassium content in grain was significantly higher in treatment T_{13} (75% NP + N_2 fixer + PSB), which was followed by T_2 was significantly higher over the respective control. Likewise, the potassium content in straw was also maximum in T_{13} (75% NP + N_2 fixer + PSB) which was at par with T_2 and T_{11}. Moreover, least values was obtained in control in both grain and straw respectively. The wheat grain contained high amount of N followed by K and P, whereas, straw was higher in K content followed by N and P. Similar reports of increase in nutrient content by PGPR formulation has been reported by [5], [20]. Researchers have reported five methods by which PGPR improves the nutrient status of host plants, namely biological N_2 fixation, increasing nutrient availability in the rhizosphere through the process of solubilization, increasing root area, enhancing other beneficial host symbioses, and combining different modes of action. PGPR promotes the development process of plants with the production of different phytohormones like IAA, gibberellic acid and cytokinins [21]. These plant growth promoters improve the availability of nutrients (N, P, Zn and Fe) [22]. Plant growth promoting rhizobacteria (PGPR) are the basic components of a biofertilizer. Bacillus and Pseudomonas are very powerful agents for bio-inoculating crops have been reported by [23], whereas, PGPR strains such as Burkholderia, Azospirillium, Enterobacter, Azotobacter, Erwinia, Rhizobium and Flavobacterium have proved fruitful for crops [24].

CONCLUSION

The studies show that a treatment combination of 75% RDF along with *Azotobacter chroococcum* (N_2 fixer), *Pseudomonas putida* and *Bacillus polymyxa* as seed treatment was more effective than treatment with PGPR alone and 50% RDF. It can enhance plant height, leaf area, dry weight, RWC and nutrient uptake in wheat. This microbial consortium may be used as efficient PGPR for wheat production in farmer's field. It is an environment friendly and cost-effective technology.

Table 3 Effect of plant growth promoting rhizobacteria consortium on nitrogen (N), phosphorus (P) and potassium (K) content in grain and straw of wheat (*Triticum aestivum* L.) at harvest during Rabi 2019 and Rabi 2020 (Pooled data)

Treatments	Nitrogen		Phosphorus		Potassium	
	Grain	Straw	Grain	Straw	Grain	Straw
T_1: Control (no fertilizers and no PGPR)	1.37	0.30	0.134	0.065	0.37	0.93
T_2: 100% NPK	1.58	0.42	0.237	0.103	0.51	1.37
T_3: N_2 fixer (*Azotobactor*)	1.40	0.32	0.142	0.070	0.38	0.94
T_4: PSB (*Pseudomonas* + *Bacillus polymyxa*)	1.39	0.31	0.148	0.071	0.39	0.72
T_5: N_2 fixer (*Azotobactor*) + PSB (*Pseudomonas* + *B. polymyxa*)	1.41	0.33	0.156	0.074	0.41	1.04
T_6: 50% NP	1.44	0.34	0.163	0.076	0.42	1.07
T_7: 50% NP + N_2 fixer (*Azotobactor*)	1.47	0.36	0.169	0.080	0.43	1.14
T_8: 50% NP + PSB (*Pseudomonas* + *Bacillus polymyxa*)	1.46	0.35	0.174	0.080	0.45	1.15
T_9: 50% NP + N_2 fixer (*Azotobactor*) + PSB (*Pseudomonas* + *Bacillus polymyxa*)	1.48	0.37	0.180	0.085	0.46	1.16
T_{10}:75% NP	1.49	0.39	0.200	0.087	0.47	1.21
T_{11}: 75% NP + N_2 fixer (*Azotobactor*)	1.53	0.41	0.213	0.095	0.48	1.26
T_{12}: 75% NP + PSB (*Pseudomonas* + *Bacillus polymyxa*)	1.51	0.40	0.227	0.104	0.49	1.22
T_{13}: 75% NP + N_2 fixer (*Azotobactor*) + PSB (*Pseudomonas* + *Bacillus polymyxa*)	1.74	0.43	0.247	0.117	0.52	1.39
S. Em (±)	0.01	0.01	0.004	0.001	0.01	0.05
C.D. at 0.5%	0.03	0.02	0.011	0.003	0.03	0.14

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